

AMENDMENT TO THE SPECIFICATION

Please replace the paragraph beginning on page 17, beginning at line 9 with the following paragraph:

Another suitable open reading frame (ORF) useful in phylogenetic analyses comprises the ORF encoding the L protein. When an overall amino acid identity of at least 91%, preferably of at least 95% of the analysed L-protein with the L-protein of isolate I-2614 is found, the analysed virus isolate comprises a preferred MPV isolate according to the invention. In analogy to other negative strand viruses, the last ORF of the MPV genome is the RNA-dependent RNA polymerase component of the replication and transcription complexes. The L gene of MPV encodes a 2005 aa protein, which is 1 residue longer than the APV-A protein (Table 5). The L protein of MPV shares 64% homology with APV-A, 42-44% with RSV, and approximately 13% with other paramyxoviruses (Table 6). Poch *et al.* (1989; 1990) identified six conserved domains within the L proteins of non-segmented negative strand RNA viruses, from which domain III contained the four core polymerase motifs that are thought to be essential for polymerase function. These motifs (A, B, C and D) are well conserved in the MPV L protein: in motifs A, B and C: MPV shares 100% similarity with all pneumoviruses and in motif D MPV shares 100 % similarity with APV and 92% with RSV's. For the entire domain III (aa 625- 847 in the L ORF), MPV shares 83% identity with APV, 67-68% with RSV and 26-30% with other paramyxoviruses (Figure 15). In addition to the polymerase motifs the pneumovirus L proteins contain a sequence which conforms to a consensus ATP binding motif K(X)₂₁GEGAGN(X)₂₀K (SEQ ID NO: 105) (Stec, 1991). The MPV L ORF contains a similar motif as APV, in which the spacing of the intermediate residues is off by one: K(x)₂₂GEGAGN(X)₁₉ K (SEQ ID NO: 106).

Please replace the paragraph beginning on page 28, beginning at line 33 with the following paragraph:

Fig. 3 Comparison of the N (SEQ ID NO: 1-7), P (SEQ ID NO: 8-13), M (SEQ ID NO: 14-20) and F (SEQ ID NO: 21-27) ORF's of members of the subfamily *Pneumovirinae* and virus isolate 00-1. The alignment shows the amino acid sequence of the complete N (SEQ ID NO: 1), P (SEQ ID NO: 8), M (SEQ ID NO: 14) and F (SEQ ID NO: 21) proteins and partial L proteins (SEQ ID NO: 28 and SEQ ID NO: 32) of virus isolate 00-1. Amino acids that differ between isolate 00-1 and the other viruses are shown, identical amino acids are represented by periods, gaps are represented as dashes. Numbers correspond to amino acid positions in the proteins. Accession numbers used for the analyses are described in the materials and methods section. APV-A, B or C: Avian Pneumovirus type A (SEQ ID NO: 2,

SEQ ID NO: 9, SEQ ID NO: 16, SEQ ID NO: 22, SEQ ID NO: 29, SEQ ID NO: 33), B (SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 23) or C (SEQ ID NO: 4, SEQ ID NO: 10, SEQ ID NO: 17, SEQ ID NO: 24), b-or hRSV: bovine (SEQ ID NO: 5, SEQ ID NO: 11, SEQ ID NO: 18, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 34) or human (SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 19, SEQ ID NO: 26, SEQ ID NO: 31, SEQ ID NO: 35) respiratory syncytial virus, PVM: pneumonia virus of mice (SEQ ID NO: 7, SEQ ID NO: 13, SEQ ID NO: 20, SEQ ID NO: 27). L8: fragment 8 obtained with RAP-PCR located in L, L9/10: consensus of fragment 9 and 10 obtained with RAP-PCR, located in L. For the P alignment, no APV-B sequence was available from the Genebank, For the L alignment only bRSV, hRSV and APV-A sequences were available.

Please replace the paragraph beginning on page 30, line 1 with the following paragraph:

Fig. 6A: Nucleotide (SEQ ID NO: 36) and amino acid (SEQ ID NO: 37, SEQ ID NO: 8, SEQ ID NO: 14, SEQ ID NO: 21) sequence information from the 3' end of the genome of MPV isolate 00-1. ORF's are given. N: ORF for nucleoprotein; P: ORF for phosphoprotein; M: ORF for matrix protein; F: ORF for fusion protein; GE: gene end; GS: gene start.

Please replace the paragraph beginning on page 30, line 6 with the following paragraph:

Fig. 6B and C: Nucleotide and amino acid sequence information from obtained fragments in the polymerase gene (L) of MPV isolates 00-1. Positioning of the fragments in L is based on protein homologies with APV-C (accession number U65312). The translated fragment 8 (Fig. 6B) (SEQ ID NO: 38 and SEQ ID NO: 39) is located at amino acid number 8 to 243, and the consensus of fragments 9 and 10 (Fig. 6C) (SEQ ID NO: 40 and SEQ ID NO: 41) is located at amino acid number 1358 to 1464 of the APV-C L ORF.

Please replace the paragraph beginning on page 30, line 20 with the following paragraph:

Alignment of the predicted amino acid sequence of the nucleoprotein of MPV (SEQ ID NO: 1) with those of other pneumoviruses (SEQ ID NO: 4, SEQ ID NO: 3, SEQ ID NO: 2, SEQ ID NO: 42, SEQ ID NO: 6, SEQ ID NO: 5, SEQ ID NO: 7). The conserved regions identified by Barr (1991) are represented by boxes and labeled A, B, and C. The conserved region among pneumoviruses (Li, 1996) is shown gray shaded. Gaps are represented by dashes, periods indicate the positions of identical amino acid residues compared to MPV.

Please replace the paragraph beginning on page 30, line 27 with the following paragraph:

Amino acid sequence comparison of the phosphoprotein of MPV (SEQ ID NO: 8) with those of other pneumoviruses (SEQ ID NO: 10, SEQ ID NO: 43, SEQ ID NO: 9, SEQ ID NO: 44, SEQ ID NO: 12, SEQ ID NO: 11, SEQ ID NO: 13). The region of high similarity (Ling, 1995) is boxed, and the glutamate rich region is grey shaded. Gaps are represented by dashes and periods indicate the position of identical amino acid residues compared to MPV.

Please replace the paragraph beginning on page 31, line 2 with the following paragraph:

Comparison of the deduced amino acid sequence of the matrix protein of MPV (SEQ ID NO: 14) with those of other pneumoviruses (SEQ ID NO: 17, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 45, SEQ ID NO: 19, SEQ ID NO: 18, SEQ ID NO: 20). The conserved hexapeptidesequence (Easton, 1997) is grey shaded. Gaps are represented by dashes and periods indicate the position of identical amino acid residues relative to MPV.

Please replace the paragraph beginning on page 31, line 8 with the following paragraph:

Alignment of the predicted amino acid sequence of the fusion protein of MPV (SEQ ID NO: 21) with those of other pneumoviruses (SEQ ID NO: 24, SEQ ID NO: 23, SEQ ID NO: 22, SEQ ID NO: 46, SEQ ID NO: 26, SEQ ID NO: 25, SEQ ID NO: 27). The conserved cysteine residues are boxed, N-linked glycosylation sites are underlined, the cleavage site of F0 is double underlined, the fusion peptide, signal peptide and membrane anchor domain are shown grey shaded. Gaps are represented by dashes and periods indicate the position of identical amino acids relative to MPV.

Please replace the paragraph beginning on page 31, line 16 with the following paragraph:

Comparison of amino acid sequence of the M2 ORFs of MPV with those of other pneumoviruses. The alignment of M2-1 ORFs is shown in panel A (SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54), with the conserved amino terminus (Collins, 1990; Zamora, 1999) shown grey shaded. The three conserved cysteine residues are printed bold face and indicated by #. The alignment of M2-2 ORFs is shown in panel B (SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62). Gaps are represented by dashes and periods indicate the position of identical amino acids relative to MPV.

Please replace the paragraph beginning on page 31, line 24 with the following paragraph:

Amino acid sequence analyses of the SH ORF of MPV. (A) Amino acid sequence of the SH ORF of MPV (SEQ ID NO: 63), with the serine and threonine residues grey shaded, cysteine residues in bold face and the hydrophobic region double underlined. Potential N-linked glycosylation sites are single underlined. Numbers indicate the positions of the basic amino acids flanking the hydrophobic domain. (B) Alignment of the hydrophobicity plots of the SH proteins of MPV, APV-A and hRSV-B. The procedure of Kyte and Doolittle (1982) was used with a window of 17 amino acids. Arrows indicate a strong hydrophobic domain. Positions within the ORF are given on the X-axis.

Please replace the paragraph beginning on page 32, line 2 with the following paragraph:

Amino acid sequence analyses of the G ORF of MPV. (A) Amino acid sequence of the G ORF of MPV (SEQ ID NO: 64), with serine, threonine and proline residues grey shaded, the cysteine residue is in bold face and the hydrophobic region double underlined. The potential N-linked glycosylation sites are single underlined. (B) Alignment of the hydrophobicity plots of the G proteins of MPV, APV-A and hRSV-B. The procedure of Kyte and Doolittle (1982) was used with a window of 17 amino acids. Arrows indicate the hydrophobic region, and positions within the ORF are given at the X-axis.

Please replace the paragraph beginning on page 32, line 11 with the following paragraph:

Comparison of the amino acid sequences of a conserved domain of the polymerase gene of MPV (SEQ ID NO: 65) and other paramyxoviruses (SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75). Domain 111 is shown with the four conserved polymerase motifs (A, B, C, D) in domain 111 (Poch 1998, 1999) boxed. Gaps are represented by dashes and periods indicate the position of identical amino acid residues relative to MPV. hPIV3: human parainfluenza virus type 3; SV: sendai virus; hPIV-2: human parainfluenza virus type 2; NDV: New castle disease virus; MV: measles virus;; nipah: Nipah virus.

Please replace the paragraph beginning on page 32, line 30 with the following paragraph:

Noncoding sequences of hMPV isolate 00-1. (A) The noncoding sequences between the ORFs and at the genomic termini are shown in the positive sense. From left to right, stop codons of indicated ORFs are shown, followed by the noncoding sequences, the gene start signals and start codons of the indicated subsequent ORFs. Numbers indicate the first

position of start and stop codons in the hMPV map. Sequences that display similarity to published gene end signals are underlined and sequences that display similarity to UAAAAAU/A/C are represented with a line above the sequence (SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84). (B) Nucleotide sequences of the genomic termini of hMPV (SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90). The genomic termini of hMPV are aligned with each other and with those of APV. Underlined regions represent the primer sequences used in RT-PCR assays which are based on the 3' and 5' end sequences of APV and RSV (Randhawa *et al.*, 1997; Mink *et al.*, 1991). Bold italicized nucleotides are part of the gene start signal of the N gene. Le: leader, Tr: trailer.

Please replace the paragraph beginning on page 33, line 19 with the following paragraph:

Amino acid alignment of the nucleoprotein of two prototype hMPV isolates (SEQ ID NO: 1, SEQ ID NO: 91).

Please replace the paragraph beginning on page 33, line 22 with the following paragraph:

Amino acid alignment of the phosphoprotein of two prototype hMPV isolates (SEQ ID NO: 8, SEQ ID NO: 92).

Please replace the paragraph beginning on page 33, line 25 with the following paragraph:

Amino acid alignment of the matrix protein of two prototype hMPV isolates (SEQ ID NO: 14, SEQ ID NO: 93).

Please replace the paragraph beginning on page 33, line 28 with the following paragraph:

Amino acid alignment of the fusion protein of two prototype hMPV isolates (SEQ ID NO: 21, SEQ ID NO: 94).

Please replace the paragraph beginning on page 33, line 31 with the following paragraph:

Amino acid alignment of the M2-1 protein of two prototype hMPV isolates (SEQ ID NO: 47, SEQ ID NO: 95).

Please replace the paragraph beginning on page 33, line 34 with the following paragraph:

Amino acid alignment of the M2-2 protein of two prototype hMPV isolates (SEQ ID NO: 55, SEQ ID NO: 96).

Please replace the paragraph beginning on page 34, line 3 with the following paragraph:

Amino acid alignment of the short hydrophobic protein of two prototype hMPV isolates (SEQ ID NO: 63, SEQ ID NO: 97).

Please replace the paragraph beginning on page 34, line 7 with the following paragraph:

Amino acid alignment of the attachment glycoprotein of two prototype hMPV isolates (SEQ ID NO: 64, SEQ ID NO: 98).

Please replace the paragraph beginning on page 34, line 11 with the following paragraph:

Amino acid alignment of the N-terminus of the polymerase protein of two prototype hMPV isolates (SEQ ID NO: 99, SEQ ID NO: 100).

Please replace the paragraph beginning on page 46, line 3 with the following paragraph:

To generate PCR fragments spanning gaps A, B and C between the RAP-PCR fragments (Fig.2) we used RT-PCR assays as described before on RNA isolated from virus isolate 00-1. The following primers were used: For fragment A: TR1 designed in the leader: (5'- AAAGAATTCACGAGAAAAAACGC-3') (SEQ ID NO: 107) and N1 designed at the 3' end of the RAP-PCR fragments obtained in N (5'-CTGTGGTCTCTAGTCCCACTTC-3') (SEQ ID NO: 108). For fragment B: N2 designed at the 5' end of the RAP-PCR fragments obtained in N: (5'-CATGCAAGCTTATGGGGC-3') (SEQ ID NO: 109) and M1 designed at the 3'end of the RAP-PCR fragments obtained in M: (5'-CAGAGTGGTTATTGTCAGGGT-3') (SEQ ID NO: 110). For fragment C: M2 designed at the 5'end of the RAP-PCR fragment obtained in M: (5'-GTAGAACTAGGAGCATATG-3') (SEQ ID NO: 111) and F1 designed at the 3'end of the RAP-PCR fragments obtained in F: (5'-TCCCCAATGTAGATACTGCTTC-3') (SEQ ID NO: 112). Fragments were purified from the gel, cloned and sequenced as described before.

Please replace the paragraph beginning on page 46, line 19 with the following paragraph:

For the amplification and sequencing of parts of the N, M, F and L ORFs of nine of the MPV isolates, we used primers N3 (5'-GCACTCAAGAGATACCCTAG -3') (SEQ ID NO: 113) and N4 (5'-AGACTTTCTGCTTTGCTGCCTG-3') (SEQ ID NO: 114), amplifying

a 151 nucleotide fragment, M3 (5'-CCCTGACAATAACCACTCTG-3') ([SEQ ID NO: 115](#)) and M4 (5'-GCCAACTGATTTGGCTGAGCTC-3') ([SEQ ID NO: 116](#)) amplifying a 252 nucleotide fragment, F7 (5'-TGCACTATCTCCTCTTGGGGCTTTG-3') ([SEQ ID NO: 117](#)) and F8 (5'-TCAAAGCTGCTTGACACTGGCC-3') ([SEQ ID NO: 118](#)) amplifying a 221 nucleotide fragment and L6(5'-CATGCCCCACTATAAAAGGTCAG-3') ([SEQ ID NO: 119](#)) and L7 (5'-CACCCCAGTCTTTCTTGAAA-3') ([SEQ ID NO: 120](#)) amplifying a 173 nucleotide fragment respectively. RT-PCR, gel purification and direct sequencing were performed as described above. Furthermore, probes used were:

Probe used in M: 5'-TGC TTG TAC TTC CCA AAG-3' ([SEQ ID NO: 121](#))

Probe used in N: 5'-TAT TTG AAC AAA AAG TGT-3' ([SEQ ID NO: 122](#))

Probe used in L: 5'-TGGTGTGGGATATTAACAG-3' ([SEQ ID NO: 123](#))

Please replace the paragraph beginning on page 51, line 18 with the following paragraph:

Primers used for diagnostic PCR:

In the nucleoprotein: N3 (5'-GCACTCAAGAGATACCCTAG -3') ([SEQ ID NO: 124](#)) and N4 (5'- AGACTTTCTGCTTTGCTGCCTG-3') ([SEQ ID NO: 125](#)), amplifying a 151 nucleotide fragment. In the matrixprotein: M3 (5'-CCCTGACAATAACCACTCTG-3') ([SEQ ID NO:126](#)) and M4 (5'-GCCAACTGATTTGGCTGAGCTC-3') ([SEQ ID NO: 127](#)) amplifying a 252 nucleotide fragment. In the polymerase protein: L6 (5'-CATGCCCCACTATAAAAGGTCAG-3') ([SEQ ID NO: 128](#)) and L7 (5'-CACCCCAGTCTTTCTTGAAA-3') ([SEQ ID NO: 129](#)) amplifying a 173 nucleotide fragment. Other primers can be designed based on MPV sequences, and different buffers and assay conditions may be used for specific purposes.

Please replace the paragraph beginning on page 52, line 5 with the following paragraph:

Oligonucleotides used for analysing the 3'end of the genome (absence of NS1/NS2).

Primer TR1 (5'-AAAGAATTCACGAGAAAAAACGC-3') ([SEQ ID NO: 130](#)) was designed based on published sequences of the trailer and leader for hRSV and APV, published by Randhawa (1997) and primer NI (5'-CTGTGGTCTCTAGTCCCACTTC-3') ([SEQ ID NO: 131](#)) was designed based on obtained sequences in the N protein. The RT-PCR assay and sequencing was performed as described above. The RT-PCR gave a product of approximately 500 base pairs which is too small to contain information for two ORFS, and translation of these sequences did not reveal an ORF.

Please replace the table beginning on page 82, line 9 with the following table:

Virus		primers	located in protein
HPIV-1	Fwd	5'-TGTTGTCGAGACTATTCCAA-3' (<u>SEQ ID NO: 132</u>)	HN
	Rev	5'-TGTTG(T1A)ACCAGTTGCAGTCT-3' (<u>SEQ ID NO: 133</u>)	
HPIV-2	Fwd	5'-TGCTGCTTCTATTGAGAAACGCC-3' (<u>SEQ ID NO: 134</u>)	N
	Rev	5'-GGTGACPT TC(T1C)AATAGGGCCA-3' (<u>SEQ ID NO: 135</u>)	
HPIV-3	Fwd	5'-CTCGAGGTTGTCAGGATATAG-3' (<u>SEQ ID NO: 136</u>)	HN
	Rev	5'-CTTTGGGAGTTGAACACAGTT-3' (<u>SEQ ID NO: 137</u>)	
HPIV-4	Fwd	5'-TTC(A1G)GTTTTAGCTGCTTACG-3' (<u>SEQ ID NO: 138</u>)	N
	Rev	5'-AGGCAAATCTCTGGATAATGC-3' (<u>SEQ ID NO: 139</u>)	
Mumps	Fwd	5'-TCGTAACGTCTCGTGACC-3' (<u>SEQ ID NO: 140</u>)	SH
	Rev	5'-GGAGATCTTTCTAGAGTGAG-3' (<u>SEQ ID NO: 141</u>)	
NDV	Fwd	5'-CCTTGGTGAiTCTATCCGIAG-3' (<u>SEQ ID NO: 142</u>)	F
	Rev	5'-CTGCCACTGCTAGTTGiGATAATCC-3' (<u>SEQ ID NO: 143</u>)	
Tupaia	Fwd	5'-GGGCTTCTAAGCGACCCAGATCTTG-3' (<u>SEQ ID NO: 144</u>)	N
	Rev	5'-GAA'MTCCTTATGGACAAGCTCTGTGC-3' (<u>SEQ ID NO: 145</u>)	
Mapuera	Fwd	5'-GGAGCAGGAACTCCAAGACCTGGAG-3' (<u>SEQ ID NO: 146</u>)	N
	Rev	5'-GCTCAACCTCATCACATACTAACCC-3' (<u>SEQ ID NO: 147</u>)	
Hendra	Fwd	5'-GAGATGGGCGGGCAAGTGCGGCAACAG-3' (<u>SEQ ID NO: 148</u>)	N
	Rev	5'-GCCTTTGCAATCAGGATCCAAATTTGGG-3' (<u>SEQ ID NO: 149</u>)	
Nipah	Fwd	5'-CTGCTGCAGTTCAGGAAACATCAG-3' (<u>SEQ ID NO: 150</u>)	N
	Rev	5'-ACCGGATGTGCTCACAGAACTG-3' (<u>SEQ ID NO: 151</u>)	
HRSV	Fwd	5'-TTTGTTATAGGCATATCATTG-3' (<u>SEQ ID NO: 152</u>)	F
	Rev	5'-TTAACCAGCAAAGTGTTA-3' (<u>SEQ ID NO: 153</u>)	

Measles	Fwd	5'-TTAGGGCAAGAGATGGTAAGG-3' (<u>SEQ ID NO: 154</u>)	N
	Rev	5'-TTATAACAATGATGGAGGG-3' (<u>SEQ ID NO: 155</u>)	

Please replace the table beginning on page 82, line 34 with the following table:

General Paramyxoviridae:

Fwd	5'-CATTA AAAAGGGCACAGACGC-3' (<u>SEQ ID NO: 156</u>)	P
Rev	5'-TGGACATTCTCCGCAGT-3' (<u>SEQ ID NO: 157</u>)	

Please replace the table beginning on page 83, line 5 with the following table:

ZF1:	5'-CCCACCACCAGAGAGAAA-3' (<u>SEQ ID NO: 158</u>)
ZF4:	5'-ACCACCAGAGAGAAACCC-3' (<u>SEQ ID NO: 159</u>)
ZF7:	5'-ACCAGAGAGAAACCCACC-3' (<u>SEQ ID NO: 160</u>)
ZF10:	5'-AGAGAGAAACCCACCACC-3' (<u>SEQ ID NO: 161</u>)
ZF13:	5'-GAGAAACCCACCACCAGA-3' (<u>SEQ ID NO: 162</u>)
ZF16:	5'-AAACCCACCACCAGAGAG-3' (<u>SEQ ID NO: 163</u>)
CS1:	5'-GGAGGCAAGCGAACGCAA-3' (<u>SEQ ID NO: 164</u>)
CS4:	5'-GGCAAGCGAACGCAAGGA-3' (<u>SEQ ID NO: 165</u>)
CS7:	5'-AAGCGAACGCAAGGAGGC-3' (<u>SEQ ID NO: 101</u>)
CS10:	5'-CGAACGCAAGGAGGCAAG-3' (<u>SEQ ID NO: 102</u>)
CS13:	5'-ACGCAAGGAGGCAAGCGA-3' (<u>SEQ ID NO: 103</u>)
CS16:	5'-CAAGGAGGCAAGCGAACG-3' (<u>SEQ ID NO: 104</u>)

IN THE SEQUENCE LISTING

Please enter the Sequence Listing enclosed herewith into the application.